LABORATORY WORK INSTRUCTION FOR QUALITY CONTROL

I. WASHING AND STERILIZATION

1. All glassware and plastic ware must be cleaned using laboratory-grade detergents and water.
2. Rinse with hot water to remove all traces of residual washing compound, and finally rinse several times with distilled. Glass ware, media, sample bottles and other material may be sterilized in an autoclave at 121°C for 15 minutes.
3. Moisture present in bottles after autoclaving may be removed by placing bottles in a drying oven at 100ºC for 10 to 15 minutes. For plastic bottles loosen caps before autoclaving to prevent distortion.

Note: Pipettes can be washed using automatic washer attached to a faucet. Use tablet type or liquid detergent designed for pipette washer. Rinse in several cycle until water will clear. Pipette will undergo sterilization thru dry oven process before using.

II.STERILIZATION PROCEDURE

1. Fill autoclave with 6 liters of distilled water.
2. Open the lid and input basket (2 basket are available).
3. Input the contents in basket and close the lid.
   Caution: Fasten the lid forcefully.
4. Press “Power Key” on control panel more than a second.
5. Set the sterilizing temperature & time (Refer to control method).
6. When temperature is reached to 108°C, bell is ringing two times automatically
7. Heating is continued, and when temperature reached to pointed sterilizing temp. (121°C) sterilization start.
8. After sterilization bell ring for pointed Bell time (Vent time).
   (Bell time: 0 – 9 minutes available).
9. Confirm that ventilation of steam from bell & pressure gauge, and then open the lid slowly. (If the pressure gauge indicates 0 it is safe).
10. Open the lid and output contents with safety glove.
    After ringing, if time is passed for Auto power off time (basically 1 minute)
    Power is turned off automatically.

III. MEDIA PREPARATION

Borosilicate glass shall be used or other suitable non-corrosive equipment such as stainless steel. Glassware must be clean and free from foreign material that may contaminate media. Glassware being used should be submitted to sterilization prior to media preparation.

A. LACTOSE BROTH

MEDIA COMPOSITION

PEPTONE	5.0 gm/L
MEAT EXTRACT	3.0 gm/L
LACTOSE	5.0 gm/L
DISTILLED WATER	1 L

1. Weigh 19.5 grams of dehydrated lactose broth media.
2. Dissolve media in sterilized beaker with 1 liter distilled water.
3. Distribute 20 ml of solution into each test tube with inverted vials.

4. Sterilize solution for 15 minutes in an autoclave.

B. BGB (BRILLIANT GREEN BILE BROTH)

MEDIA COMPOSITION

PEPTONE	10.0 gm/L
LACTOSE	10.0 gm/L
OXGAIL	20.0 gm/L
BRILLIANT GREEN	0.0133 gm/L
DISTILLED WATER	1 L

1. Weigh 40 grams of media, dissolved with 1 liter of distilled water.
2. Distribute 10 ml of solution into each test tube with inverted vials.
3. Sterilize solution @ 121°C for 15 minutes in an autoclave.

C. EC BROTH

MEDIA COMPOSITION

TRYPTOSE	20.0 g
LACTOSE	5.0 g
BILE SALTS MIXTURE	1.5 g
DIPOTASSIUM HYDROGEN PHOSPHATE,K2HPO	4.0 g
POTASSIUM DIHYDROGEN PHOSPHATE,KH2PO	1.5 g
SODIUM CHLORIDE, NaCl	5.0 g
DISTILLED WATER	1.0 L

1. Weigh 37.5 grams of media in 1 liter distilled water.
2. Distribute 10 ml. of solution in test tube with inverted vials.
3. Sterilized media @ 121°C for 15 minutes in an autoclave.

IV. PREPARATION OF SODIUM THIOSULFATE SOLUTION

1. Dissolved 1.5 grams of sodium Thiosulfate with a 100 ml.of distilled water.
2. Add 0.5 ml of this solution in each sampling bottle.
3. Sterilize the bottle for 15 minutes @ 121°C in an autoclave.

V. PREPARATION OF SAMPLING BOTTLE

1. Sample bottle for bacteriological testing must have a wide mouth opening and with a capacity of 120 ml.
2. Wash bottles and caps with detergent and water.
3. Rinse with hot water followed by rinsing with distilled water. Allow to dry then add 0.5 ml. or 7 drops of Sodium Thiosulfate solution to de-chlorinate sample that was treated with chlorine.
4. Cover sampling bottle and sterilized in an autoclave for 15 minutes @ 121°C.
5. After sterilization, cover tightly the bottle.

VI. COLLECTION OF SAMPLE

Importance of proper sampling is a vital part of monitoring the quality of water. Major source of error is the whole process of obtaining water quality information often occurs during sampling. If sampling is performed in a careless and thoughtless manner incorrect data may result to poor management. Water sample should be conducted such a way that analytical result represents the actual sample composition. And that the sample is protected from contamination or alteration.

COLLECTION PROCEDURE:

A. FROM PUMP OR FAUCET

1. Select nearest pipe connected to a faucet.
2. Flame the tap with cotton soaked with alcohol.
3. Allow it to run to waste for about 5 to 10 minutes to permit clearing of line. Restrict the flow to avoid splashing, unscrew the cap of the sampling bottle without exposing the inside to wind and dust.
4. Fill bottle to the neck leaving airspace to facilitate mixing of sample by shaking preparatory to examination. Stopper bottle immediately.

B. SAMPLING FROM SURFACE

1. Remove the cover and hold the bottle near its base and plunge downward one foot below the surface and collect sample by forward sweep of the arm with the mouth of the bottle forward or upstream.

VII. PRESERVATION AND STORAGE OF SAMPLE

1. No preservation by ice is necessary if sample is examined within one hour after collection.
2. Sample should be preserved in ice if they are to be examined more than one hour after collection at temperature below 10ºC.
3. Time between collection and examination shall not be greater than 24 hours and not greater than 30 hours if refrigerated.

VIII. TRANSPORT OF SAMPLE

1. Fill up all space provided in Requisition Form (see Attached Form).
2. Sample without proper identification shall not be accepted.
3. Sample should be submitted from Monday to Wednesday from 8:00 AM to 1:00 PM. at Cagayan de Oro City Water District, Booster Pumping Station, Macasandig, Cagayan de Oro City.

SAMPLE LABELING

All water sampling bottle are to be labeled with a sampling sticker or paper tag as shown below:

Description/ Site :_____
Location : _____
Date :_____
Time : _____
Sampler : _____
Type of Analysis :_____

SUBMISSION OF SAMPLE

If client submit sample to the laboratory, they are going to fill up form (see attached Form) for data information. Sample is then labeled after receiving with the following information:

Laboratory Number
Date Sample Received
Analysis Requires
Initials of staff member who accepted the sample

Laboratory Sample Label
Laboratory No.

Date Received. : _____
Analysis Required: _____
Signature: _____

IX. LABORATORY HOUSEKEEPING

Housekeeping must be of high standard at all times considering safety as well as for quality of results. Laboratory is to be cleaned daily and the cleaning be recorded in a housekeeping logbook. Preparation and Sterilization of work station should be clean and free from dust during analysis considering that test is microbiological.

For Microbiological Laboratory, the following are to be followed:

I. Work benches are to be swabbed with 70 % alcohol twice daily before and after work commences.
II. Laboratory rooms, shelves, floor and windows are regularly cleaned to avoid contamination in process of analysis.
III. Floors are to be wet-mop and treated with disinfectant.

STANDARDANALYTICALPROCEDUREFORMICROBIOLOGICAL ANALYSIS

MULTIPLE TUBE FERMENTATION TECHNIC

INTRODUCTION

The standard test for the coliform group may be carried out either by the multiple tube fermentation technique or presence-absence procedure, the membrane filter technique or by the enzymatic substrate coliform test. Each technique is applicable within the limitations specified and with due consideration of the purpose of the examination.

When multiple tubes are used in the fermentation technique, results of the examination of replicate tubes and dilutions are reported in terms of the Most Probable Number (MPN) of organisms present. This number, based on certain probability formulas, is an estimate of the mean density of coliforms in the sample.

APPARATUS AND EQUIPMENT:

a. Autoclave for operation at 121°C
b. Incubator, to maintain at 35 ± 0.5°C
c. Oven
d. Biosafety Cabinet
e. Glass wares:
   Fermentation tubes, Shell Vials, Inoculating Loop, Inoculating Needle, Burner, Pipette, test tube racks, petri dish, pipettor
f. Safety Equipment:
   Laboratory Gown and Shoes, Face Mask, Hair net, Gloves
g. Colony Counter
h. Water Bath

0.01 TEST PROCEDURES FOR COLIFORM BACTERIA

PRESUMPTIVE TEST:

1. Shake sample bottle upside and down vigorously for homogenous dispersion of bacteria. Arrange fermentation tubes in rows of Five (5) each in a test tube rack.
2. For Five (5) tubes that contain 20 ml of lactose broth with inverted vials transferred 10 ml of water samples to each test tube. Gently shake tubes in rotating direction for proper mixing of samples.
3. Incubate the inoculated tubes at 35 ± 0.50°C.
4. After 24 ± 2hr of incubation swirl each tube and examine for gas formation. If no gas is presence, re-incubate and re-examine at the end of total 48 ± 3h.
5. Observe and record samples with presence or absence of gas formation. Presence of both gas and growth constitutes a positive presumptive test should be transfer for confirmatory stage.
6. Samples without gas formation after 48 hrs of incubation constitutes for negative coliform bacteria.

CONFIRMATORY TEST:

1. Subject all positive presumptive tubes to the confirmed phase.
2. Gently shake or swirl the positive tubes to re-suspend growth and with a sterile loop, transfer one loopful of a sample in a fermentation tube containing brilliant green lactose bile broth.
3. Incubate the inoculated tubes at 35 ± 0.5°C.
4. Formation of gas within 24 - 48 ± 3h after incubation constitutes a positive confirmed phase.

TABLE 1

MPN INDEX AND 95% CONFIDENCE LIMITS

No. of Tubes Giving Positive Reaction out of 5 (20 ml. each)	MPN Index / 100 ml	95% Confidence Limits (Exact) Lower	Upper
0	< 1.1	_	3.5
1	1.1	0.015	5.4
2	2.6	0.40	8.4
3	4.6	1.0	13
4	8.0	2.1	23
5	>8.0	3.4	-

a. TEST PROCEDURE FOR FECAL COLIFORM BACTERIA

The determination of fecal coliform bacteria presence is base on the tube of samples from presumptive test that produces gas formation and are subject for testing.

1. From positive samples, gently shake or swirl the tubes showing gas or acidic growth to re-suspend the organism. With a sterile loop or sterilized wooden stick, transfer one or more loopfuls of culture sample to a fermentation tube containing EC medium with an inverted vials. Repeat for all other positive presumptive tubes.
2. Incubate the inoculated tubes of samples with a temperature of 35 ± 0.5°C for 24 – 48 ± 3hrs.
3. After the incubation, record result of observation of the sample. Sample that produces gas formation signify the presence of fecal coliform bacteria.

MEMBRANE FILTER TEST METHOD

APPARATUS AND EQUIPMENT:

FILTERING DEVICE
ASPIRATOR
MEMBRANE FILTER
COMPACT DRY
STIRILZED FILTER
STERILIZED TWEEZER
STERILIZED PLASTIC PIPET
70 % ALCOHOL WIPES

PROCEDURE:

1. Prepare the Compact Dry plates, label data, and take off the cap of the plate. Pipette 1 ml of sterile purified water and diffuse in the middle of dry sheet.
2. Remove the funnel of the sterilized filtering membrane device wipes with 70 % alcohol wipes as disinfectant of the device.
3. Open a package of sterilized filter and pick up the membrane filter with sterilized tweezers.
4. Set the funnel and pour the 100 ml sample water in the funnel and filter the sample water under reduced pressure.
5. After the filtration, detached the funnel and take the membrane filter out with sterilized tweezers. Place the filtered membrane sample on the prepared Compact Dry plates without bubble and close.
6. Turn over the plate capped and put in an incubator for incubation under the prescribed condition. Incubate at 37°C for at least 18 – 24 Hrs.

INTERPRETATION OF RESULT:

COLIFORM:
Have the enzyme - ß –D – galactosidase
Can be detected with magenta – Gal artificial sugar
Color development is Pink

E – COLI:
Have the enzyme – ß - glucuronidase
Can be detected with X – Gluc artificial sugar
Developed Blue Color

RAPID DETECTION METHOD

0.1 PRESENCE / ABSENCE (P-A) COLIFORM TEST

APPARATUS AND EQUIPMENT:

1. UV LAMP (365nm)
2. 120 ml Borosilicate Graduated Glass
3. Commercially Snap Pack Reagent
4. Comparator

PROCEDURE:

1. Carefully remove snap pack from the strip, taking care not to tear adjacent pack.
2. Take snap pack of reagent open the pack by snapping back the top at the score line;shortly tap to ensure the granules are at the bottom. Bend the upper part of the snap pack until it breaks open.
   Note: Do not touch the opening to avoid contamination risk.
3. Add the reagent to a 100 ml water sample in sterile, transparent non – fluorescent vessel.
4. Aseptically cap and seal the vessel and shake until granules completely to dissolve.
5. Incubate at 18 – 24 hrs at 35°C to 37°C
6. After incubation period observed, record result

INTERPRETATION OF RESULTS FOR TOTAL COLIFORMS / E.COLI:

Read the results at 18 hours but not later than 24 hours. Compare each result to a comparator.

- If no colour is observed or changed, the sample is negative for coliform and E. Coli.
- If samples has change colour ≥ as observed in the comparator, the sample is confirmed positive for coliform.
- If colour changed is observed, check vessel for flourescene by placing a 6 watt 365 nm UV light from the samples in a dark environment. If sample flouresence is ≥ to the comparator the sample is confirmed positive for E. Coli.
- Be sure the light is facing away from eyes.

LABORATORY WORK INSTRUCTION FOR QUALITY CONTROL

HETEROTROPHIC PLATE COUNT

INTRODUCTION

The Heterotrophic Plate Count (HPC), formerly known as the Standard Plate Count, is used to count non-specific bacteria in water. Colonies may arise from pairs, chains, clusters, or single-cells, all of which are included in the term " colony-forming units ".Heterotrophic organisms are any organism that obtains nutrients by feeding off of the compounds in the water. *Heterotrophs* are organisms, including bacteria, yeasts, and moulds, that require an external source of organic carbon for growth. The method may be used to monitor changes in the bacteriological quality of finished water throughout a distribution system, thus giving an indication of the effectiveness of water treatment. The HPC Test is the internationally accepted test for measuring the heterotrophic microorganism population in drinking water.

Although most, if not all, bacterial pathogens are heterotrophs, most of the microorganisms detected by the HPC Test conditions are not human pathogens, thus the colony counts obtained do not alone normally correlate with the presence of pathogens, in the absence of other indicators of fecal contamination.

There are three different methods for performing the heterotrophic plate count - the pour plate, the spread plate, and the membrane filtration.

The Pour Plate Method is the one most commonly used because it is simple to perform and can accommodate volumes of sample or diluted sample ranging from 0.1 to 2.0 ml. The colonies produced are relatively small and compact, showing fewer tendencies to encroach on each other than those produced by surface growth.

PURPOSE

To estimate the number of live heterotrophic bacteria in water.

DEFINITION OF TERMS

1. HPC – Heterotrophic Plate Count
2. CFU – colony-forming unit
3. Culture media – contains the needed nutrients that support the growth of microorganisms.

MATERIALS & EQUIPMENTS

1. Biological Safety Cabinet
2. Autoclave
3. Incubator, capable of maintaining 35± 1°C
4. Quebec colony counter
5. Analytical balance
6. Magnetic stirrer
7. Hot plate
8. Sterile graduated pipettes, pipetting aide, pipette canisters
9. Petri dishes
10. Dilution bottles, borosilicate-resistant glass
11. Media bottle
12. Stir bars
13. Flask or Beaker
14. Weighing boats

MEDIA& REAGENTS (POUR PLATE METHOD)

1. Plate Count Agar (PCA)

Tryptone	5.0 g
Glucose	1.0 g
Yeast Extract	2.5 g
Agar	15.0 g
Reagent –grade water	1.0 L

2. Distilled Water (Sterilized)

PREPARATION OF PCA

1. Suspend 22.5 g in 1 L distilled water.
2. Mix and melt PCA using hotplate/magnetic stirrer.
3. Transfer rehydrated PCA for plates into media bottle. Cover with appropriate closures.
4. Autoclave for 15 min at 121°C at 15 psi.

SAMPLE PREPARATION

1. Mix samples or dilutions thoroughly by rapidly making about 25 complete up-and-down (or back and forth) movements.

PLATING & INCUBATION OF SAMPLE

1. Pipette 1ml and 0.1ml of undiluted water sample into appropriately marked sterile Petri dishes.
2. Pipette 1ml of undiluted water sample and add to 99 ml sterilized distilled water. From this dilution, pipette 1ml and 0.1 ml into appropriately marked sterile Petri dishes.
3. Pour at least 10-12 ml Plate Count Agar to each dish. No more than 20 min. should elapse between dilution of the sample and pouring of agar into the last dish.
4. As each plate is poured mix melted medium thoroughly with test portions in Petri dish by rotating the dish in one direction or by rotating and tilting.
5. Let plates solidify.
6. Invert plates and incubate for 48 hr at 35°C.
7. Count the number of colonies per plate as “Colony Forming Unit “.

Note: Dilution to be used depends on the sample source. For treated waters, direct testing of 0.1 or 1.0 ml sample may be done. If the source is suspected to be contaminated, or if the sample is taken directly from a surface water, then a 100x or 1000x dilution may be needed

COUNTING & RECORDING

1. **For plates with 30-300 colonies.** When counts of plates fall within and outside the 30-300 colony range, use only those counts that fall within the range.

$$\text{CFU/ ml} = \frac{\text{total number of colonies counted}}{\text{Actual volume of sample, ml}}$$

2. **No plate with 30-300 colonies, and one or more plates have more than 300 colonies**. Use the plate(s) having a count nearest 300. Compute the count as in step 1.

3. **Plates with no colonies**. When plates from all dilutions have no colonies, report the count as less than one (<1) divided by the corresponding largest sample volume used.

4. **All plates with more than 300 colonies**. When plates from all dilutions yield more than 300 colonies, do not report result as "Too Numerous To Count" (TNTC). Count colonies in 13 squares (of the colony counter). If possible, select seven consecutive squares horizontally across the plate and six consecutive squares vertically. Multiply sum of the number of colonies in the 13 representative square centimetres by 5 to compute estimated colonies per plate when plate area is 65 cm^2.

REPORTING

HPC results are expressed as cfu/ml.
Philippine National Standards for Drinking Water (PNSDW) limit :< 500 cfu/ml.

I. MEMBRANE FILTER COMPACT DRY METHOD

Compact Dry is ready to use test method which help to reduce the time needed to perform microbial testing. It allows maximum productivity by increasing efficiency. The sample is self diffuse evenly over the whole plate. The growth colonies are pigmented and identified by its colour. Colonies developed by chromogenic substrates and redox indicator.

PROCEDURE :

1. Select a compact Dry HP plate, remove the plate cap and pipette 1 ml of sterile water into the middle of the dry sheet.
2. Using sterile tweezers pick up a sterile membrane filter
3. Remove the funnel from a sterilized filtering device and set the sterilized membrane filter.
4. Set the funnel, pour the sample water into the funnel and filter the sample water under reduced pressure.
5. After filtering the sample, wash the inner surface of the funnel with 20 – 30 ml of sterile water and filter it. Repeat the same steps two or three times.
6. Detach the funnel and take the membrane filter out with sterilized tweezers. Put the filter on the prepared Compact Dry Hp plate avoiding any bubbles. The trap side is upper.
7. Turned over the capped plate and put in an incubator under the prescribed condition.

A. Compact Dry AQ
Heterotrophic Plate Count

Procedure:

1. Open the cap and drop 1 ml of specimen on the middle of the Compact Dry Plate.
2. Specimen diffuses automatically and evenly into the sheet and transforms the dried sheet into a gel within seconds.
3. Put the cap again on the plate and write the information needed on the memorandum section.
4. Turn over the capped plate and put in the incubator in a horizontal position.
5. After the incubation, count the number of all grown colonies underneath the plate. White paper placed under the plate helps count the colonies or use a colony counter machine.

Incubation Time:

36 $^oC \pm 2\ ^oC$ for 44 $\pm$ 4h
22 $^oC \pm 2\ ^oC$ for 68 $\pm$ 4h(ISO 6222)

35 ^{o}C ± 1 ^{o}C for 48 ± 4h (Filter/SMEWW Method)

Interpretation of the Results:

Colonies grown are almost all red.
Yeasts tend to grow as white-pink.
Molds grow as typical 3-dimensional cottony form.

B. Compact Dry EC

E.*coli* and coliforms

Procedure:

1. Open the cap and drop 1 ml of specimen on the middle of the Compact Dry Plate.
2. Specimen diffuses automatically and evenly into the sheet and transforms the dried sheet into a gel within seconds.
3. Put the cap again on the plate and write the information needed on the memorandum section.
4. Turn over the capped plate and put in the incubator in a horizontal position.
5. After the incubation, a blue colony growth is indicative of E.*coli* and the total coliform group count is the sum of both the red and blue colonies.

Incubation Time:

37 ^{o}C ± 1° C for 24 ± 2
35 ^{o}C ± 1 ^{o}C for 24 ± 2h (Filter/SMEWW Method)

I. DETERMINATION OF CHLORINE RESIDUAL

EQUIPMENT AND APPARATUS:

1. CHLORINE RESIDUAL TEST KIT
   INSTRUMENT TYPE: COLORIMETER METHOD
   STANDARD: STANDARD METHOD 4500 –Cl –G
   UNIT of MEASUREMENT: PARTS PER MILLION (ppm)
   MILLIGRAM PER LITER (mg/l)
   RANGE: 0.00 – 10ppm free and TOTAL CHLORINE
   RANGE SELECTION: AUTOMATIC
   RESOLUTION: 0 – 5ppm RANGE

2. REAGENTS :
   CHLORINE DPD # 1 TABLET
   CHLORINE DPD # 3 TABLET
   CHLORINE DPD 1A LIQUID
   CHLORINE DPD 1B LIQUID
   CHLORINE DPD 3 LIQUID

3. WATER SAMPLE BOTTLE 60 ml.
4. TUBES
5. CABLE, USB 3 ft.
6. USB ADAPTOR

CHLORINE ANALYSIS PROCEDURE:

0.1 CHLORINE - FREE & TOTAL - TABLET REAGENT

1. Press meterto turn On.
2. Select measure.
3. Select Free Chlorine.
4. Rinse Tube ,Fill with sample, Dry Tube.
5. Scan Blank.
6. Add Chlorine DPD Tablet # 1, Shake 10 second; Invert slowly 5 times, Dry Tube.
7. Scan Free Chlorine.
8. Record Free chlorine result.
9. Add Chlorine DPD # 3 Tablet, Shake 10 seconds, Invert slowly 5 times, Dry Tube.
10. Scan Total Chlorine.
11. Read and Record Total Chlorine result.
12. Total Chlorine – Free Chlorine = Chlorine combined.
13. Press meter to turn Off.

0.2 CHLORINE – FREE & TOTAL – LIQUID REAGENTS

1. Press meterto turn On.
2. Select measure.
3. Select Free Chlorine.
4. Rinse Tube, Fill with Sample, Dry tube.
5. Scan Blank.
6. Add 5 drops Chlorine DPD 1A and 5 drops Chlorine DPD 1B Mix, Dry Tube.
7. Scan Free Chlorine.
8. Read and Record Free Chlorine result.
9. Add 5 drops Chlorine DPD 3, Mix, Dry tube.
10. Scan Total Chlorine.
11. Record Total Chlorine result.
12. Total Chlorine – Free Chlorine = Combined Chlorine.
13. Press to turn meter Off.

Equipment: Picco Colorimeter
Serial Number: 12131325
Manufacturer: Spectoquant - Merck

CALIBRATION

1 After the instrument has been switched off, hold the **[SELECT]** and **[ZERO/TEST]** keys depressed. 2 Switch the instrument on using the **[ON/OFF]** key, after approx. 1 second release the **[SELECT]** and **[ZERO/TEST]** keys.
3 Press the **[MENU]** key to select the menu item **"Cal".**
4 Confirm the selection with the **[SELECT]** key.
5 The display shows in alternating mode **CAL**
6 Scroll through methods using the **[SELECT]** key.
7 Conduct zero setting
7.1 Fill a clean cell with a blank sample, close with the cell cap, and place in the measurement compartment with the cell mark pointing to the mark of the instrument casing.
7.2 Press the **[ZERO/TEST]** key.
7.3 Method symbol blinks for approx. 8 seconds.
7.4 The display shows: **0.0.0**
7.5 Remove the cell from the measurement compartment after completing the zero setting.
8 Press the **[ZERO/TEST]** key.
9 Method symbol blinks for approx. 3 seconds.
10 The display shows in alternating mode **CAL**

11 Position the cell with the standard solution in the measurement compartment.
12 Press the **[ZERO/TEST]** key.
13 Method symbol blinks for approx. 3 seconds.
14 The display shows in alternating mode: **CAL**
15 If - in consideration of the tolerance - the result matches the value of the standard, exit the adjustment mode by pressing the **[ON/OFF]** key.
16 Pressing the **[SELECT]** key once raises the result by 1 digit. Pressing the **[ZERO/TEST]** key once reduces the result by 1 digit.
17 Repeatedly press keys until the displayed result matches the value of the standard.
18 Press the **[ON/OFF]** key to calculate the new correction factor and to save it at the user adjustment level.
19 The display shows (confirmation of adjustment: **: :**
20 Recommended adjustment settings: for all methods, the middle of the measuring range.

Equipment: SpectroquantPicco
Method: Photometry
Test: Residual Chlorine (U.2: Cl2)

Preparation:

- Analyze immediately after sampling
- The pH must be within the range 4-8
- Filer turbid samples

Procedure:

1 Press **"on"** to start
2 Select **U.2** on the Selection Series
3 Once selected, prepare the equipments.
4 Pour **10mL** water sample on provided bottle.
5 Add and mix **6 drops reagent Cl_2-1** and **3 drops of reagent Cl_2-2**. Leave to stand for **1 min**.
6 Wipe the bottle for excess water.
7 Put the container in the sample well facing the "" in the front.
8 Press the zero/test and wait for the result.
9 Copy and repeat test if needed.

Equipment: SpectroquantPicco
Method: Photometry
Test: Chlorine Dioxide (U.4: ClO2)

Preparation:

- Analyze immediately after sampling
- The pH must be within the range 4-8
- Filer turbid samples

Procedure:

1 Press **"on"** to start
2 Select **U.4** on the Selection Series
3 Once selected, prepare the equipments.
4 Pour **10mL** water sample on provided bottle.
5 Add **2 drops reagent ClO_2-1** and mix. Leave to stand for **2 mins**.
6 Add **reagent ClO_2-2, 1 level** blue microspoon.
7 Add and shake vigorously until the reagent is completely dissolved. Leave to stand for **1 min**.
8 Wipe the bottle for excess water.

9 Put the container in the sample well facing the " " in the front.
10 Press the zero/test and wait for the result.
11 Copy and repeat test if needed

Equipment: Hach CLD-1 (28428-00)
Method: Colorimetry
Test: Chlorine Dioxide

Procedure:

1 Fill a viewing tube to the first 5 mL line with sample water. This is the blank Note: Complete the test and read the result within 1 minute after adding powder.
2 Place the test tube in the top left opening of the color comparator.
3 Fill another viewing tube to the first 5 mL line with sample water. This is the sample.
4 Add 2 drops of Glycine Reagent to the sample tube. Swirl to mix.
5 Add the contents of one DPD Free Chlorine Reagent Powder Pillow to the sample tube.
6 Swirl to mix. A pink color will develop if chlorine dioxide is present.
7 Place the sample tube in the top right opening of the color comparator.
8 hold the comparator up to a light source. Look through the openings in front.
Note: Acceptable light sources include the sky, a window, or a lamp.
9 Rotate the color disc until the color matches in the two openings.
10 Read the mg/L chlorine dioxide in the scale window.

II. DETERMINATION OF TURBIDITY

APPARATUS & EQUIPMENT:

1. TURBIDIMETER
2. BLANK TUBES
3. CALIBRATION STANDARD
4. LINT FREE CLOTH

EQUIPMENT CALIBRATION:

1. Press meter to turn On.
2. Select measure.
3. Select Turbidity with blank.
4. Rinse tube three times with 0 NTU Standard or Turbidity – free water
   Fill the tube to the line with 0 NTU Standard or Turbidity – free water.
   Cap the tube. This is the BLANK.
   Tip: Use a clean, smudge-free, scratch-free tube. Do not use a tube or cap that was used for high turbidity standards.
5. Wipe the tube thoroughly with a lint- free cloth
   Tip: Surround the tube with a clean, lint-free cloth. Press the cloth around the tube and rotate the tube three times in the cloth to assure that all areas of the tube have been wiped.
6. Insert the tube into the chamber. Close the lid. Scan blank. Remove the tube.
   Tip: Align the index line on the tube with the index arrow on the meter.
   Tip: After scanning the blank, scan the blank again as a sample. It should be read 0.00, If not, re-blank the meter and scan the blank again. Repeat until it read is 0.00. A small negative number will be observed if the reading is slightly less than the reading used as the blank. This is expected due to minute variation between readings.
7. Empty the tube. Rinse the same tube three times with the 1 NTU standards.
   Fill the tube to the line with 1 NTU Standard. Cap the tube.

Tip: For the most accurate results, the same tube should be used for the blank, 1 NTU Standard and the Sample to eliminate error caused by tube to tube variation.

Tip: Fill the tube slowly, pouring down the inside wall of the tube to avoid into producing bubbles.

8. Wipe the tube thoroughly with a lint-free cloth
9. Insert the tube into the chamber. Close the lid. Scan Sample
   Tip: Scan the sample three times, removing the tube from the chamber after each scan. The reading should be consistent. Use the last consistent reading to calibrate the meter.
10. Press V , Select Calibrate
11. Press Λ or V to change the turbidity reading on the display to read 1.000
12. Press enter to set calibration
13. Proceed to Analysis

TURBIDITY TEST PROCEDURE :

1. Press meterto turn On.
2. Select measure.
3. Select turbidity with blank.
4. Rinse the tube three times with 0 NTU Standard or free turbidity water. Fill tube to the line with 0 NTU or free turbidity water. Cap the tube and this is the blank.
5. Insert the tube into the chamber. Close the lid. Scan Blank. Remove the tube.
6. Empty the tube. Rinse the same tube three times with the sample. Fill tube with a sample up to the line then cap the tube.
7. Wipe the tube thoroughly with a lint free cloth
8. Insert the tube into the chamber. Close the lid. Scan Sample.
9. Read and Record the result

III. DETERMINATION OF pH IN WATER

The basic principle of electromagnetic pH measurement is the determination of the activity of the hydrogen ions by potentiometric measurement using a standard hydrogen electrode and a reference electrode. The instrument is calibrated using two buffers and its performance is checked using a third buffer. Samples must be dilute aqueous simple solutions (<0.2M). Determination of pH cannot be made accurately in non-aqueous media, suspensions, colloids, or high-ionic-strength solutions.

Instrument Calibration:

1. Before use, remove the glass electrode from the storage solution, rinse with deionised water, and blot dry with soft tissue or cloth.
2. Calibrate the pH meter with the pH 7 buffer using the standard operation procedure.
3. Make preliminary reading of sample.
4. If pH is < 7, set slope using pH 4 and pH 7 buffers. If pH > 7, set slope with pH 7 and pH 9.22 buffers (Refer to Operational SOP for pH meter,

Sample Analysis:

1. Remove electrode from buffer, rinse with deionised water and rinse with sample solution to be measured, blot dry, and place in test solution/sample.
2. Establish equilibrium between electrodes and sample by stirring the sample to insure homogeneity; stir gently using a stirrer to minimise CO2 entrapment. Press measure.

3. Record pH reading when READY sign appears. Record two more readings of the same sample by repeating step 2pH.

Equipment: Starter 3100 Bench pH Meter
Serial Number: B410355531
Manufacturer: OHAUS Corp.

CALIBRATION INFORMATION
There are 3 buffer groups in the meter, you can select the buffer group you prefer, default buffer are pH 4.01, pH 7.00 and pH 10.01; the buffer value will be automatically recognized during calibration. The 3 predefined buffer groups are (at 25^{o}C). STARTER 3100 automatically corrects for the temperature dependence of the buffer pH values given in the following table – buffer group.

ONE-POINT CALIBRATION
1 Place the pH electrode in a calibration buffer, stir 5 seconds, wait for 10-15 seconds.
2 Press button-**CAL** "*Cal* 1" displays on the bottom left on the screen and "*Cal"* is blinking. Cal and appear on the top of the screen, is blinking during calibration.
3 The meter reaches **endpoint** according to the **endpoint mode,** the calibration point pH value (e.g. 7.00) with the temperature display on the screen.
4 The 1-point calibration is finished; now we have 3 choices to do next: Press button-**Cal** to do the 2-point calibration. Press button-**Read** to store 1-point calibration and exit, the **offset** and the **slope** are shown on the display for 3 seconds then return to the measurement screen. Press button-**Exit** to reject the calibration, return to the measurement screen.

TWO-POINT CALIBRATION
1 Perform the 1-point calibration as described above.
2 Rinse the pH electrode with pure water and wipe off with tissue.
3 Place the electrode in the next calibration buffer, stir and wait, then press button-**Cal**, "*Cal* 2" displays on the bottom left of the screen and "*Cal*" is blinking. On the top of the screen Cal and and appear on the top of the screen, is blinking during calibration.
4 The meter reaches endpoint according to the endpoint mode, the calibration point pH value (e.g. 4.01) with the temperature display on the screen.
5 The 2-point calibration is finished; now we have 3 choices to do next: Press button-**Cal** to do the 2-point calibration.
Press button-**Read** to store 1-point calibration and exit, the **offset** and the **slope** are shown on the display for 3 seconds then return to the measurement screen.
Press button-**Exit** to reject the calibration, return to the measurement screen.

Equipment: OHAUS Meter
Method: Glass Electrode
Test: pH and Temperature

Reminder:
- Plug the adaptor every morning during shift and unplug after shift.

Procedure:
1 Press the start button.
2 Put the sampling container on the electrode and then press "auto".
3 Wait for a few seconds for the result then record the result.
4 Repeat procedure if more than one sample is tested.

LABORATORY WORK INSTRUCTION FOR QUALITY CONTROL

Equipment: pH 700 Benchtop Meter Manual
Method: Glass Electrode
Test: pH and Temperature

pH Calibration

For best results, periodic calibration with known accurate standards is recommended. Calibrate with standards that bracket your intended measuring range while including a neutral standard (pH 7.00 or 6.86). For example, if you expect to measure samples from pH 6.2 to pH 9.5, calibration with 4.01, 7.00, and 10.01 will work well.

The 700 series meters can be calibrated with up to 5 buffers. The non-volatile memory retains all calibration values upon meter shut down. The following calibration standards are automatically recognized; USA buffer group 1.68, 4.01, 7.00, 10.01, 12.45 NIST buffer group 1.68, 4.01, 6.86, 9.18, 12.45 See Section 5.6 to change the buffer group To eliminate temperature errors associated with the pH electrode, attach the automatic temperature compensation (ATC) probe for best accuracy. Without temperature compensation, pH accuracy will worsen as samples deviate from 25ºC and pH 7.

1 Press as needed to select pH.
2 Dip the pH and ATC electrodes into pH buffer and press . The secondary display will lock on the appropriate buffer value. Provide stirring for best results. When the READY indicator appears, press to accept. The primary reading will flash briefly before the secondary display begins scrolling the remaining available buffers.
3 Rinse the pH and ATC electrodes then dip into the next pH buffer. The secondary display will lock on the appropriate buffer value. When the READY indicator appears, press to accept. The primary reading will flash briefly then display the percent efficiency (slope) before the secondary display begins scrolling the remaining available buffers.
4 To calibrate another buffer repeat step 3 or press to return to the measurement mode.
Note: The meter will automatically return to measurement mode upon successful completion of the number of specified calibration points. To specify a different number of pH calibration points see Section 5.6.

Additional notes: A single point (offset) calibration is only allowed with pH 7.00 or pH 6.86 buffers. When the first calibration value is accepted during a new calibration, all prior calibration values are erased. Press at any time to abort calibration and return to measurement mode.

Temperature Calibration

The thermistor sensor used for automatic temperature compensation and measurement is both accurate and stable, so require frequent calibration isn't required. Temperature calibration is recommended upon electrode replacement, whenever the temperature reading is suspect, or if matching against a certified thermometer is desired.

1 Connect the temperature probe to the meter and place into a solution with a known accurate temperature such as a constant temperature bath. Note: To adjust the manual temperature compensation (MTC) value, do not connect the temperature probe.
2 Press as needed to select pH or mV/R.mV.
3 Press followed by . The primary display shows the measured temperature while the secondary display shows the factory default temperature.
4 Adjust the temperature using or . Press to accept or to cancel. The meter allows an adjustable maximum value of ± 5ºC (or ± 0.9ºF) from the factory default temperature.

FLUSHING

Objectives - To test hydrants to make sure adequate flow and pressure is available. Flushing is also done to remove sediments from the pipes in order to maintain water clarity and quality in the distribution pipes.

The Cagayan de Oro City Water District (COWD) conducts flushing based on the following:

1. Duly approved Flushing Schedule
2. Complaints of Dirty Water
3. Flushing After Repair

Tools needed:

1. Pipe Wrench
2. T-Wrench
3. Hole Digger
4. Bar
5. Shovel
6. Pressure Gauge
7. Turbidimeter
8. Residual Chlorine Test Kit

Procedure:

1. Determine the pressure in the area using pressure gauge, water pressure should be 7 meters or above.
2. Open the blow-off valve at the end points.
3. After Ten (10) Minutes or more, determine the turbidity and residual chlorine of the water. Turbidity should not be greater than 5 NTU. Residual chlorine should not be lower than 0.3 PPM. These values are based on the Philippine National Standards for Drinking Water (PNSDW).
4. Close the blow-off valve.
5. Quarterly routine of flushing in all the blow-off points.
6. For complaints of dirty water from concessionaires, flushing will be done directly on the meter.
7. Flushing after repair will be done after each repair in the specified area.

PUMP TENDERING

Objective –

This procedure addresses the operation of the pumping facilities of **CAGAYAN DE ORO CITY WATER DISTRICT** in order to provide water supply to the customers 24 hours a day.

Work Instructions –

1. The Pump Operator shall monitor the operation of the facilities for continues supply of water.

2. The Pump Operator shall monitor that all motors are operational.
   The collector wells are at normal levels.
   The controllers/censors are functioning.
   The surroundings are clean and tidy.
   Monitor the operation of the pump motors at 30 minutes interval. The following information are to be recorded:
   a. Flow
   b. Totalizer Reading
   c. Pressure
   d. Chlorine Residual
   e. Sump Level
   f. On/Off of Motor Controllers

   Note: If the device used to measure any of the above is not operational, report to the Immediate Supervisor and record for Corrective Action.

3. Monitor 1.50-meter water level of the collector well within the range at 30 minutes' interval.
   If below, reduce the motor speed using the Variable Frequency Drive (VFD).
   If above 3.00 meter, increase the motor speed using the Variable Frequency Drive (VFD).

4. Record all activities for Monthly Operation Report.

CLEANING AND DISINFECTION

Objectives - To conduct cleaning and disinfection of sumps and reservoir to eliminate, remove and deactivate pathogenic bacteria that maybe transmitted to water

The Cagayan de Oro City Water District (COWD) conducts cleaning and disinfection based on the following:

1. Recurring microbiological problem
2. After Repair ,Emergency incidents or as needed
3. Duly approved Cleaning and Disinfection Schedule

Tools needed:

1. Pipe Wrench
2. T-Wrench
3. Hole Digger
4. Bar
5. Shovel
6. Pressure Gauge
7. Turbidimeter
8. Residual Chlorine Test Kit

Procedure:

1. Determine the pressure in the area using pressure gauge, water pressure should be 7 meters or above.
2. Open the blow-off valve at the end points.
3. After Ten (10) Minutes or more, determine the turbidity and residual chlorine of the water. Turbidity should not be greater than 5 NTU. Residual chlorine should not be lower than 0.3 PPM. These values are based on the Philippine National Standards for Drinking Water (PNSDW).
4. Close the blow-off valve.
5. Quarterly routine of flushing in all the blow-off points.
6. For complaints of dirty water from concessionaires, flushing will be done directly on the meter.
7. Flushing after repair will be done after each repair in the specified area.